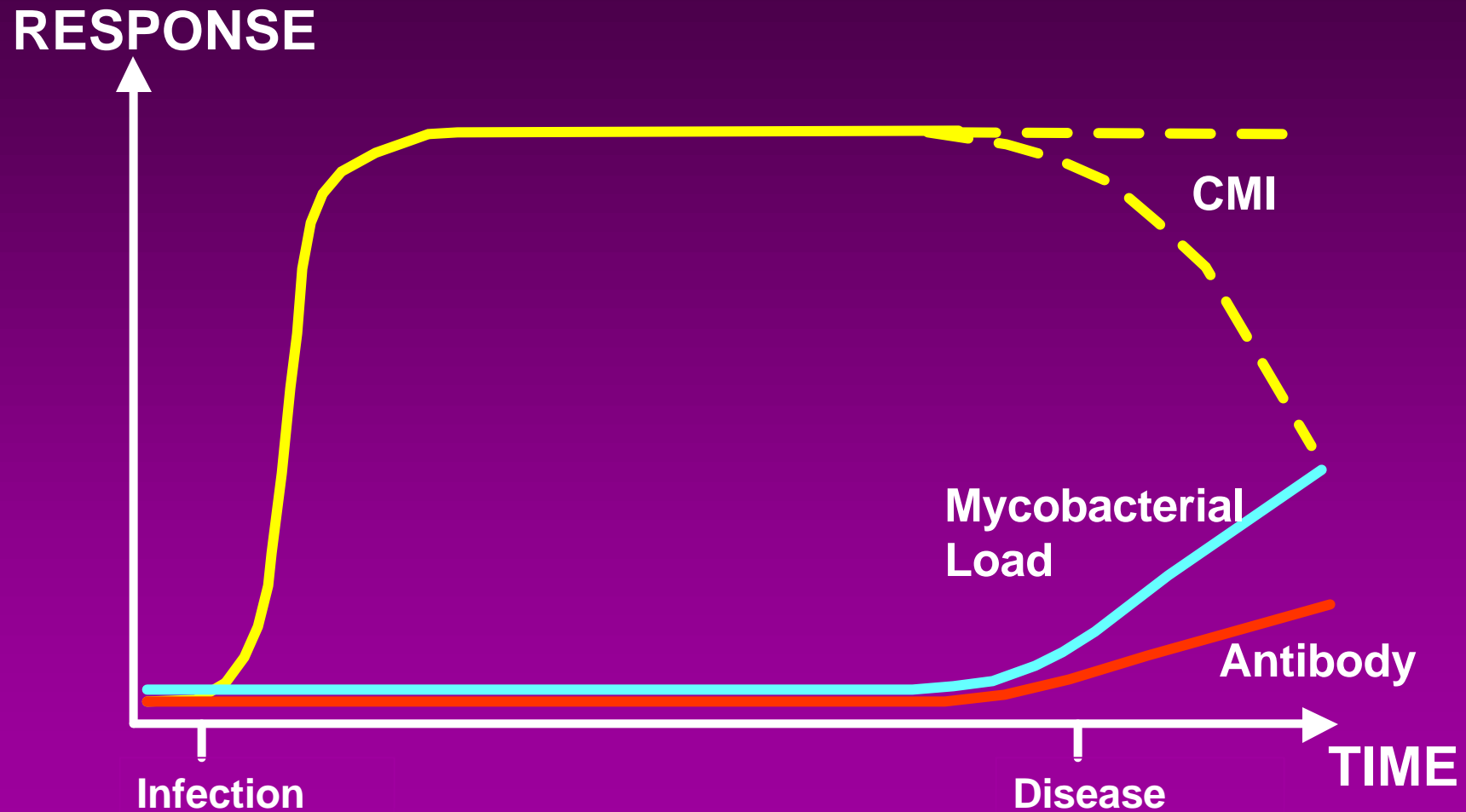# QuantiFERON-TB

Randall Reves

Major Problems with the TST

- **TST responses are often not read**
  - Patient and public health implications
  - Cost implications (follow up and re-testing)

- **False positive TST due to**
  - BCG
  - NTM

- **Inaccuracy of measuring induration**
  - Subjective interpretation
  - Conscious or unconscious bias

History of the QuantiFERON-TB Test

- Interferon-γ test developed in the late 1980's for detection of TB in cattle

- Pre-clinical and initial clinical studies conducted in Australia to set test parameters and cut-offs

- Large-scale, pivotal clinical studies conducted by the CDC and the Walter Reed Army Institute of Research

- FDA approval granted in November 2001

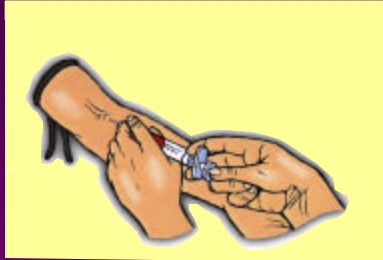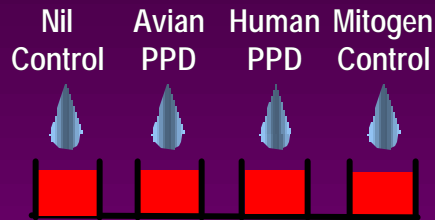# Immune Response to TB Infection and Disease

Why Measure Interferon-γ

- IFN- γ ≡ CMI

- Antigen specific

- Secreted, measurable, stable

- Absent from normal circulation

- Extensive literature showing importance of IFN-γ in TB infection
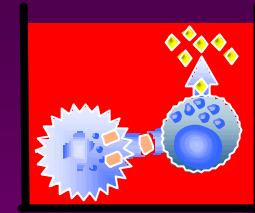
*Quanti*FERON®-TB Test Method

Stage 1 Whole Blood Culture

Heparinised whole blood

Nil Control   Avian PPD   Human PPD   Mitogen Control

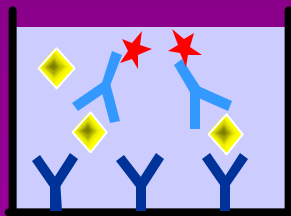**Transfer undiluted whole blood into wells of a culture plate and add antigens**

Culture overnight at 37°C

TB infected individuals respond by secreting IFN-g

Stage 2 IFN-gamma ELISA

Harvest Plasma from above settled cells and incubate 60 min in 'Sandwich' ELISA

COLOR

TMB
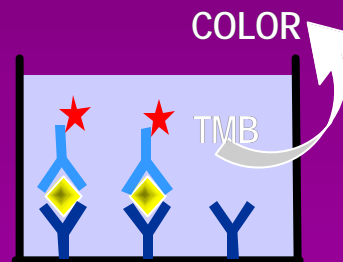
Wash, add Substrate, incubate 30 min then stop reaction

Standard Curve

OD 450nm
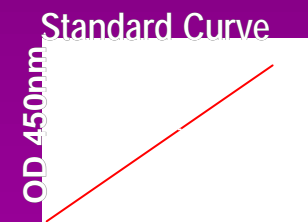
Measure OD and determine IFN-glevels

Test Interpretation

Test Result	Nil	HuPPD	AvPPD	Mitogen
Negative	–	–	–	+++
MTB Infection indicated	–	+++	+	+++
Atypical mycobacteria	–	+	+++	+++
Indeterminate	–	–	–	–

Compare *Quanti*FERON®-TB with TST in a high-risk group

- No "gold standard for LTBI"
- Remember TST is our "Bronze standard"
- Test *utility* in high risk LTBI subjects
- Objective of QFT/TST comparison?
  - Should be similar *but not identical*
  - Ideally should be better
  - Do differences tell us anything?

QFT (QuantiFERON-TB Test)
Vs.
TST (Tuberculin Skin Test)
Study

- Sponsored by CDC
- Published in JAMA 2001;286:1740-1747

Study Objectives

- Assess agreement & concordance between QFT and TST

- Identify factors associated with discordance

Enrollment

- **Enrolled 1,471 at 5 sites minus 248 excluded**
  - **98** "low risk" for exposure
  - **944** "high risk" for LTBI
  - **94** "TB suspects"
  - **87** "culture-confirmed TB" after Rx

Results

- 341 of 1223 (28%) QFT positive

- 390 of 1223 (32%) TST positive

- 84% agreement overall

Low-Risk Subjects

- **TST X QFT**

			QFT	
			Mtb	not Mtb
TST	positive	Count	0	2
		% of Total	0 %	2.0 %
	negative	Count	2	94
		% of Total	2.0 %	95.9%

Kappa= NA

- **Agreement = 95.9%**
- **Assuming no LTBI**
  - QFT "specificity" = 98.0%
  - TST "specificity" = 98.0%

CDC QFT study of high risk subjects

QFT+

TST-,QFT+
71

TST+,QFT+
146

TST -

TST+

TST-,QFT-
648

TST+,QFT –
79

QFT-
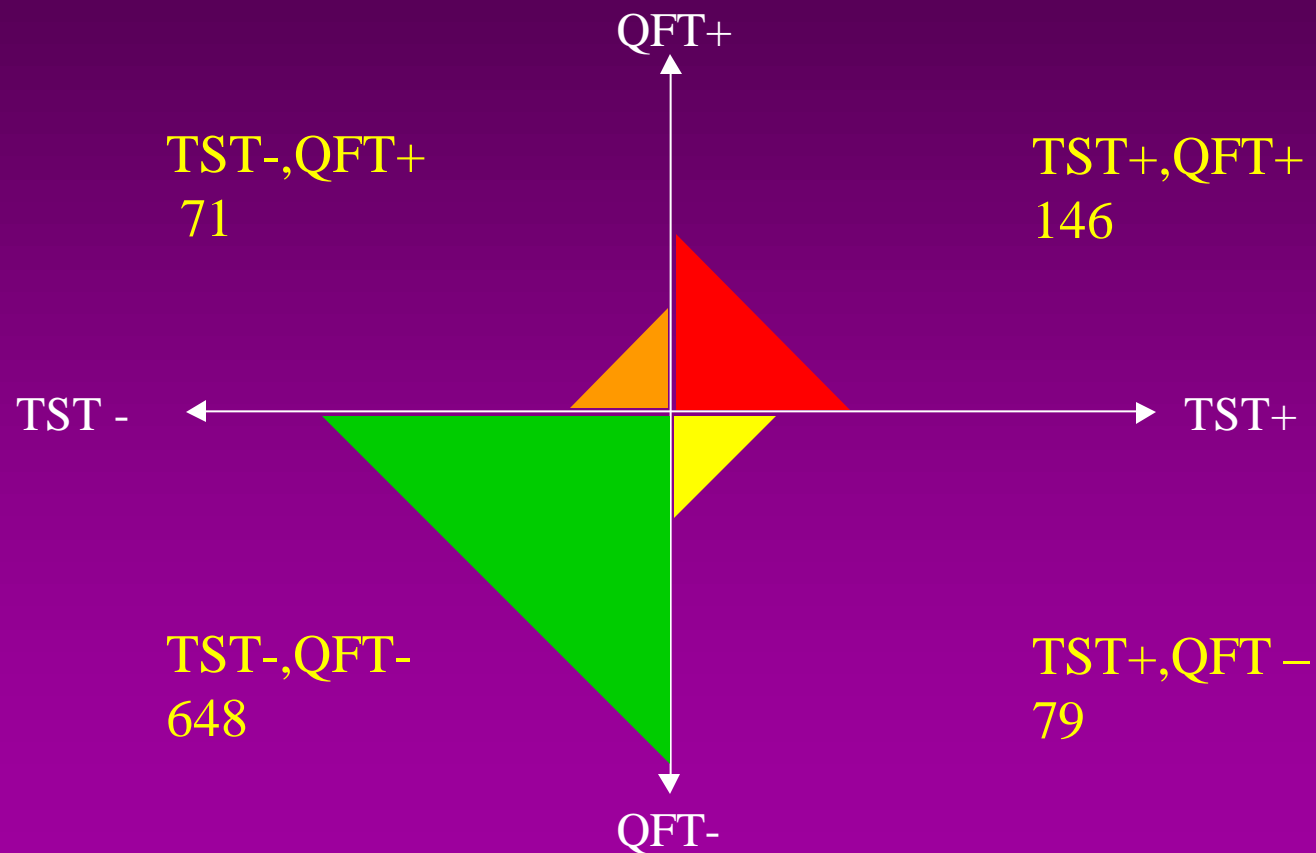
Kappa 0.56, agreement 84.1%

TST+ / QFT- Discord
(High-Risk Group)

VARIABLE		Odds Ratio	p value
BCG	None	1.0	
	Unknown	2.37	.05
	Vaccinated	6.48	< .001
NTM by QFT	No	1.0	
	Yes	2.47 (12)	.02
Study Site	A	1.0	
	B	1.56	.48
	C	3.46	.03
	D	4.23	.01
	E	3.50	.03

Discordance in TST - Tubersol and Aplisol

Low Risk (n = 1555):

		Aplisol		
		+	--	
Tubersol	+	10	3	
	--	18	1524	kappa = 0.482

Current TB disease (n = 99):

		Aplisol		
		+	--	
Tubersol	+	82	6	
	--	4	7	kappa = 0.526

Villarino et al, JAMA 1999

TB Suspects with + Cultures for Mtb

- **TST X QFT**

			QFT Mtb	QFT not Mtb
TST	positive	Count	43	9
		% of Total	76.8 %	16.1 %
	negative	Count	3	1
		% of Total	5.4 %	1.8%

- **Agreement = 76.8%**
- QFT Sensitivity = 82.2%
- TST Sensitivity = 92.9%

After Treatment for Culture-Confirmed TB

- **TST X QFT**

			QFT	
			Mtb	not Mtb
TST	positive	Count	56	27
		% of Total	64.4 %	31.0 %
	negative	Count	0	4
		% of Total	0 %	4.6 %

- **Agreement = 69.0%**
- QFT Sensitivity = 64.4 %
- TST Sensitivity = 95.4 %

Effect of Active TB & Rx
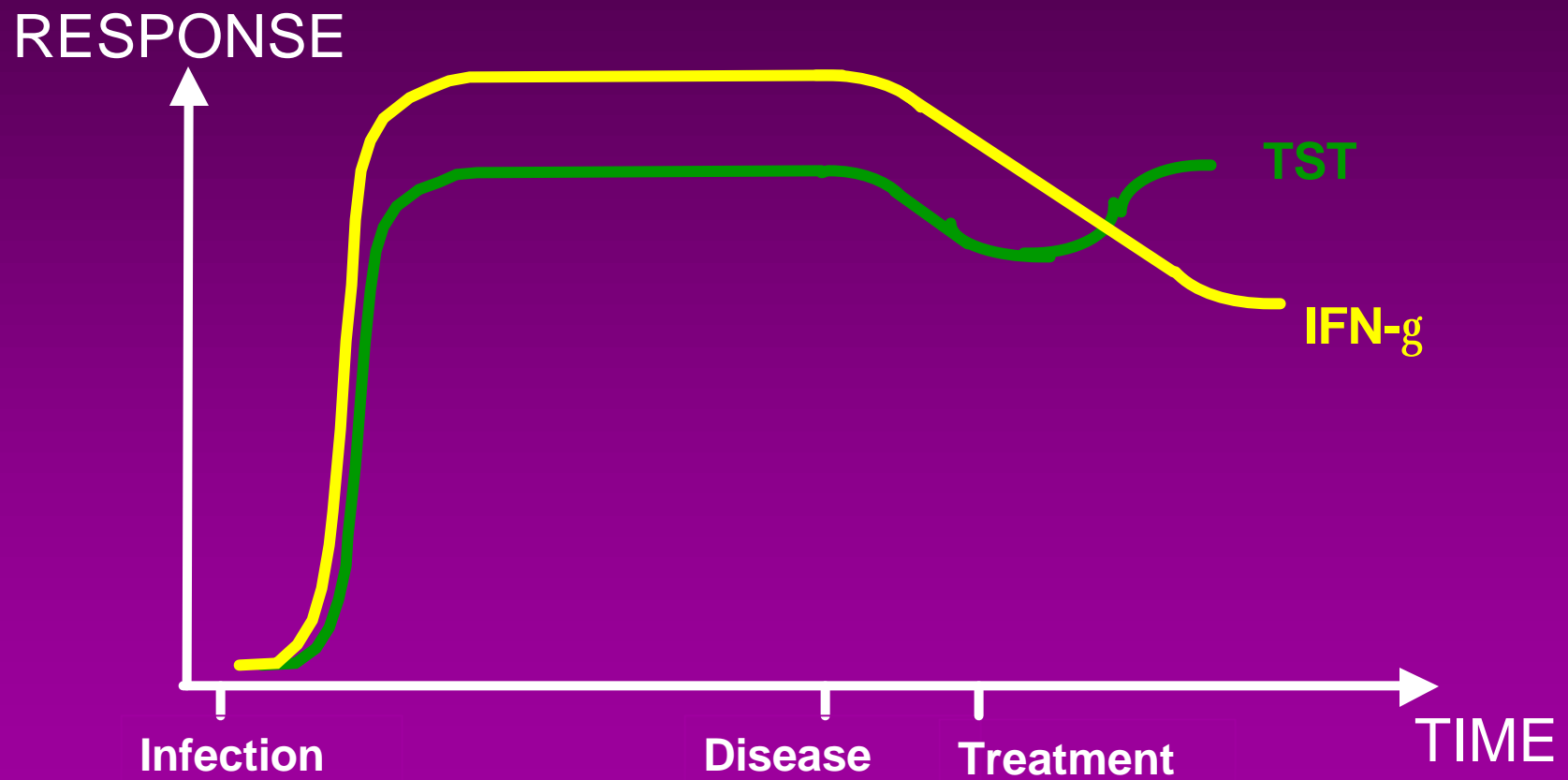
Factors Not Associated with TST / QFT Discord

- Age
- Sex
- Race
- Risk for HIV
- TST in prior year

- Delay to blood incubation for QFT
- Incubation period
- Delay to ELISA
- Time of TST reading

Limitations

- lack of a "gold standard" for Latent TB Infection
  - can not extrapolate sensitivity for LTBI from culture-confirmed TB after initiation of Rx
  - difficult to prove superiority of a test w/o follow-up

- small number of TB suspects all of which had Rx for undetermined periods

- different Mtb PPDs used for QFT and TST

- PPD from only one NTM included & no skin test for MAC included

Conclusions

- High QFT specificity in "low risk" people (98%)

- Good QFT / TST agreement for "high risk" (84%)

- QFT / TST discordance associated with:
  - Prior BCG
  - Site-bias in reading TST
  - NTM immune reactivity
  - TB Treatment

QuantiFERON-TB

Conclusions from the animal model
and clinical studies in humans

Developed in Cattle – A Good Model for Human TB
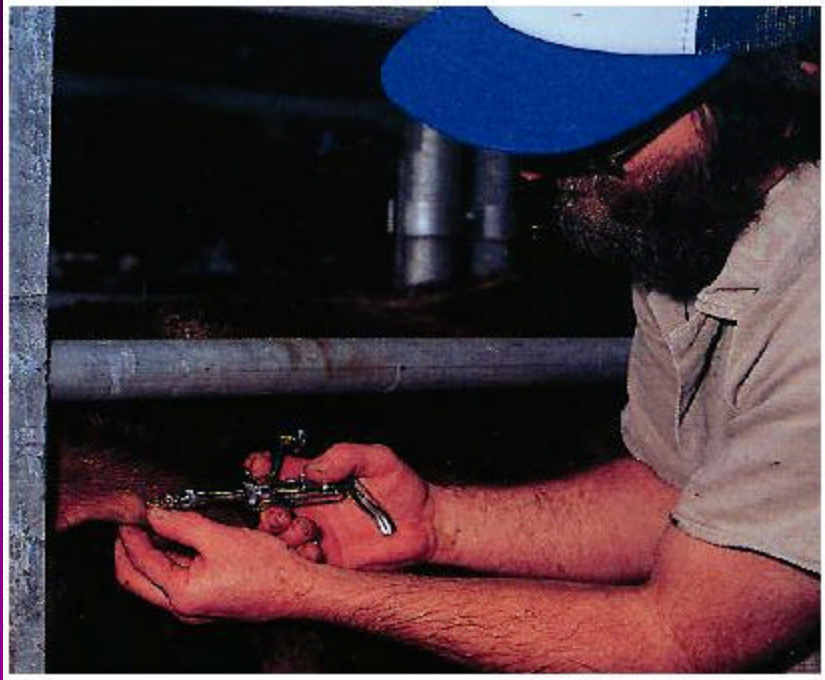
M. bovis PPD injected intradermally and read 72 hrs later

M. avium PPD is used as well for Comparative Testing

• Bovine TB is an excellent model for human TB

• Immune response to infection is very similar

• Most infected cattle have LTBI

• Active TB disease normally found only in old or undernourished animals

24

Australian Field Trial Data (1989/90)
TST comparison

Bovine IFN-gAssay

TST		+	--
	+	**92**	**53**
	--	**67**	**6090**

IF the TST was used as the gold standard:

Sensitivity of the bovine test = 92/145 (63.4%)

Australian Field Trial Data (1989/90)
Necropsy & culture as gold standard

Animals Tested from Infected Herds n=6302

Test	No. Positive	Sensitivity
TST	82	65.6%
Bovine IFN-γ	117	93.6%
TST &/or Bovine IFN-γ	119	95.2%
M. bovis culture*	125	--

* Culture was performed on multiple tissues after autopsy

Wood & Rothel, Vet Micro 40: 125-135 (1994)

Reported sensitivities for Bovine IFN-γ test

No. Tested	No. with *M. bovis*	Sensitivity	Reference
39	39	53.8 %	Ritacco et al (1991) *Argentina*
843	10	90.0 %	Ryan (1992) *New Zealand*
1362	22	81.8 %	Wood et al (1992) *Australia*
474	39	97.4 %	Dondo et al (1993) *Italy*
240	27	96.3 %	Buonavoglia et al (1995) *Italy*
1619	154	87.7 %	Domingo et al (1995) *Spain*
6210	85	85.8 %	Monaghan et al (1995) *Ireland*
208	208	96.6 %	Dondo et al (1996) *Italy*
203	203	87.7 %	Monaghan et al (1997) *Ireland*
11,198	**787**	**88.8 %**	**Total**